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Seroprevalence and Association with Disease of Feline Coronaviruses (FCoV) Serotype 1 and 2 in Switzerland

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Seroprevalence and Association with Disease of Feline Coronaviruses (FCoV) Serotype 1 and 2 in Switzerland

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Abstract

To determine the prevalence of antibodies to Feline Coronavirus (FCoV) serotypes 1 and 2 in Switzerland and their association with different disease manifestations, a serological study based on immunofluorescence tests was conducted with Swiss field cats using Transmissible Gastroenteritis Virus (TGEV), FCoV type 1 and FCoV type 2 as antigens. A total of 639 serum samples collected in the context of different studies from naturally infected cats were tested. The current study revealed that, with an apparent prevalence of 83%, FCoV serotype 1 is the most prevalent serotype in Switzerland. FCoV type 1 viruses induced higher antibody titers than FCoV type 2, and were more frequently associated with clinical signs and/or FIP. The antibody development in 7 cats experimentally infected with FCoV type 1 revealed that, with progressing duration of infection, antibodies to FCoV type 1 significantly increased over those to FCoV type 2. There was a significant relationship between antibody titers against TGEV, FCoV 1, and FCoV 2 and TGEV antigen detected the highest proportion of seropositive cats.

We conclude that a vaccine against FCoV should be based on FCoV type 1-related antigens and that for serodiagnosis of FCoV infection TGEV should be used to attain the highest diagnostic efficiency. When serology is used in addition to clinical signs, haematology, and clinical chemistry results as an aid to diagnose clinical FIP, TGEV shows an equal diagnostic efficiency as FCoV antigen.

Key words: FCoV type 1 and 2, TGEV, seroprevalence, Switzerland, FIP, cats, diagnostic efficiency

Introduction

Intensive research has been done since the first description of the disease pattern of feline infectious peritonitis (FIP) in 1963 (22), and yet the epidemiology and the pathogenesis of the fatal disease FIP which is caused by a coronavirus is still not fully understood. It was shown that FIP is the single most important infectious cause of death in young cats resulting in the loss of 10% of seropositive kittens during the first year of life (7).

As for the pathogenesis, it is generally accepted today that FCoV and FIP inducing viruses (FIPV) represent virulence variants of the same virus rather than separate virus species (40). Most FCoV mutants do not cause clinical signs, although present at high viral loads; only sporadically mutants are pathogenic and induce FIP (29). In some cases FCoV infection was found to induce mild enteric symptoms (28, 40). It was postulated earlier that harmless FCoV were restricted to the intestinal tract and that FIP development would result from the capability of a virus mutant to induce systemic infection (34). In the meantime, it was demonstrated by RT-PCR that FCoV generally induces systemic infection (11, 25, 29). Moreover, FCoV and FIPV have remained serologically and genetically indistinguishable.

Antigenetically, coronaviruses are divided into five groups. Together with Canine Coronavirus (CCoV) and Transmissible Gastroenteritis Virus (TGEV) FCoV belongs to Group I. Cats seem susceptible to all Group I coronaviruses (24). Some feline strains are thought to origin from recombinations of FCoV and other Group I viruses, such as CCoV (17).

Based on in vitro neutralization tests, FCoV were further classified into two serotypes, which differ in their growth characteristics in cell cultures and antigenetic relationship to TGEV and CCoV; however, both serotypes can cause FIP (8, 33). The serological distinction of FCoV type 1 from type 2 is most likely associated with differences in the S-gene sequence as monoclonal antibodies to the S-protein readily differentiate the FCoV subtypes (21). Furthermore, FCoV type 1 and 2 have a different cell tropism which can be explained by changes in the S-protein binding to different receptors as demonstrated (19). Cats recovering from FCoV infection develop especially high titers against the S-protein. Many new strains have recently been isolated (4, 5, 16, 18, 26, 36) and phylogenetic examination suggests a spectrum of strains ranging from very feline like to more canine like rather than two distinct serotypes (4, 5, 26).

Whichever system is applied to classify FCoV, the association between certain FCoV strains and their ability to induce disease could not yet be elucidated.

Knowledge of the serotype circulating in a given population is an important prerequisite for development of a FCoV vaccine in that the vaccine should be closely related to the field viruses. The present study was initiated to determine the seroprevalence of FCoV type 1 and 2 in Switzerland and find potential associations between the serotypes and certain disease manifestations. In addition, we aimed to characterize the immune response during experimental infection with FCoV 1.

Material and Methods

Cats and serum samples

A total of 667 serum samples collected from naturally and experimentally infected cats during different studies were tested for FCoV antibodies (Table 1).

The kinetic of antibody development was determined in samples collected from cats orally infected with FCoV-Rm, a serotype 1 strain (35). Blood had been taken weekly during the first two months and monthly thereafter up to week 42 after infection (Table 1, Group E).

To evaluate the prevalence of FCoV serotype 1 and 2 antibodies, field sera collected in 1996/97 (10) were used. From this collection, we randomly selected 296 cats considered healthy by their owners and veterinarians (Table 1, Group A). To determine the predominant FCoV serotype present in catteries, samples were used that had been collected in 21 catteries with different breeds in 2001/2002 (15) (Table 1, Group B). From each cattery, 3 randomly selected samples were pooled because of small sample volumes.

To investigate a possible relationship between the presence of antibodies to the two serotypes and clinical signs, samples from the above mentioned 296 healthy cats and 204 clinically ill cats were tested. The latter were presented to veterinarians with one or more clinical signs of disease including fever, cachexia, depression, diarrhoea, gastrointestinal tract and/or abdominal problems. The cats originated from all parts of Switzerland and were of different ages, breeds, husbandry managements, and sexes (Table 1, Group A).

To further characterize the relationship between the presence of antibodies to one of the FCoV serotypes and FIP, 30 serum samples were evaluated that had been randomly chosen from a collection of histopathologically confirmed FIP cases accumulated between 1991 and 1995 (37) (Table 1, Group C). As a reference group, we randomly selected 28 sera collected from diseased cats without FIP; these serum samples had been stored at the Clinical Laboratory of the Vetsuisse Faculty, University of Zurich, during the same time period (Table 1, Group D).

To determine the immunological relationship between FCoV 1 and 2 and TGEV, serum samples of group A were tested for the respective antibodies.

Table 1: Origin of samples and grouping for the questions to be answered.

Serum samples Questions to be answered	Group A field sera 1996/97	Group B catteries	Group C histologically confirmed FIP cases	Group D non-FIP cases*** 1991-1995	Group E experimentally infected cats
1. Determination of the seroprevalence of FCoV serotypes in Switzerland	296 healthy cats and 204	63 samples from 21 catteries **			
2. Determination of the association between FCoV types and disease/symptoms	cats with clinical signs found to be associated with disease and/or FCoV infection*		30 sera	28 sera	
3. Determination of the immunological relationship between FCoV type 1 and 2 and TGEV					
4. Determination of the kinetics of the development of antibody titres to FCoV 1 and 2					28 samples from 7 cats****

* cats showing one or more of the following symptoms: fever, cachexia, depression, diarrhoea, gastrointestinal tract and/or abdominal problems.

** 3 pooled sera per cattery.

*** Healthy cats and cats with clinical signs of non infectious origin (e.g. fracture).

**** 4 serum samples from each cat were collected on weeks 3, 12/13, 16/17, 40/42 after experimental infection with FCoV- Rm (35).

Immunofluorescence Assay (IFA)

The FCoV slides and all materials (dilution and rinse buffer, control sera, mounting fluid) were kindly donated by VMRD, Inc. (Pullman, WA, USA). Two different types of slides were used: FCoV type 1 and type 2, grown on Crandell Feline Kidney Cells (CrFK). The positive control sera had been obtained from ascites of confirmed FIP cases and titrated on both FCoV 1 and FCoV 2 slides. The negative control sera had been collected from clinically healthy cats.

Serial dilutions of 1:100, 1:400, 1:1'600, 1:6'400, 1:25'600, 1:102'400, and 1:409'600 were prepared from all samples. The first screening was done at the dilution of 1:6'400 to avoid false positive results by cross-contamination on the slides. Endpoint titers were then determined by testing the positive samples at the higher dilutions whereas the negative samples were further tested at lower dilutions.

The staining procedure for indirect IFA was performed according to the manufacturer's protocol. Briefly, diluted sera were incubated at 37°C for 30 minutes, slides were then washed for 10 minutes before the conjugate (sheep anti-feline-IgG polyclonal antiserum conjugated to fluorescein isothiocyanate) was added for 30 minutes at again 37°C. After each washing step, the surfaces of the slides were dried with absorbing paper, preventing the wells from drying out. Each well contained 30% of infected and 70% of uninfected cells, which served as internal negative control.

Serum samples in which antibodies to type 1 were higher than those to type 2 were designated as "antibodies to type 1" and vice versa.

Antibodies to TGEV were tested according to Osterhaus and Horzinek (31).

Quality control of the FCoV and TGEV slides

To test the 10-wells-slides for absence of potential viral contaminants originating from various feline pathogens handled in our laboratory, cells were removed from the slides using a sterile scalpel blade and suspended in 200 µl Phosphate Buffered Saline (PBS) 1x. Total nucleic acids (TNA) were extracted using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, Rotkreuz, Switzerland) and eluted in 100 µl elution buffer. The extracts were tested by real-time RT-PCR and PCR for the following pathogens: FCoV (13), feline leukaemia virus (FeLV provirus PCR (38)), feline immunodeficiency virus (FIV) (27), feline herpes virus 1 (FHV-1) (41), feline parvo virus (FPV) (29), and feline calicivirus (FCV) (14). FCV was tested with the following modifications: forward primer 5'-GTT GGA TGA ACT ACC CGC CAA TC-3'; reverse primer 5'-CAT ATG CGG CTC TGA TGG CTT GAA ACT G-3'; probe 5'-FAM-TCG GTG TTT GAT TTG GCC TG-6-TAMRA-3' (C. Helps, personal communications). As uninfected cells present on the slides, serving as internal negative control, did not show any unspecific fluorescence, the slides were not tested for mycoplasma contamination by PCR.

Molecular characterization of the FCoV 1 and 2 isolates

To further characterize the FCoV strains used for IFA, infected cells were lysed directly on the slides using 500 µl RLT buffer and viral RNA was extracted by means of the RNeasy Mini Kit (Qiagen, Hombrechtikon, Switzerland). The extracted RNA was checked for FCoV by real time PCR (13). A region of about 700 base pairs of the S-gene of FCoV was amplified using the following primer sets: primer set 1 with forward primer UCD1.3502f 5'-GCA CTT AAT GCT TAT GTG TCT CAA A-3' and reverse primer UCD1.4165r 5'-TGA GCC ATT CAA GGT CAA CA-3'; primer set 2 with forward primer UCD1.3500f 5'-CAG CAC TTA ATG CTT ATG TGT CTC A-3' and reverse primer UCD1.4173r 5'-CAA TCC TGT TGA GCC ATT CAA G-3'; primer set 3 with forward primer FIPV79-1164.3549f 5'-AGC ACT TAA TGC ATT TGT GTC TCA-3' and reverse primer FIPV79-1164.4230r 5'-TTT CAA TTC TAT TGA GCC ATT CAA G-3'; primer set 4 with forward primer KU2.3523f 5'-GCA CTT AAT GCT TAT GTG TCT C-3' and reverse primer KU2.4226r 5'-CAC ACA TAC CAA GGC C-3'; primer set 5 with forward primer KU2.3522f 5'-AGC ACT TAA TGC TTA TGT GC-3' and reverse primer KU2.4226r (sequence above). Briefly, 2.5 µl RNA were amplified using the SuperScript™ III One-Step RT-PCR System with Platinum® Taq Kit (Invitrogen life technologies, Basel, Switzerland), 0.2 µM of each primer, in a total volume of 25 µl. PCR cycling conditions were as follows: 30 min at 50°C (RT step), 2 min at 94°C, 40 cycles of 15 sec at 94°C (denaturation), 30 sec at 55°C (annealing) and 1 min at 68°C (elongation), followed by 5 min at 68°C.

PCR products from conventional PCR were analyzed on 2% agarose gels; amplicons were purified using the MinElute Gel Extraction Kit (Qiagen) and sequenced from both sides. Cycle sequencing was performed with approximately 20 ng of DNA and 3.3 pmol product-specific primers using the BigDye Terminator Cycle Sequencing Ready Reaction Kit v1.1 (Applied Biosystems, Rotkreuz, Switzerland). Cycling conditions were as follows: 1 min at 96°C, then 25 cycles at 96°C for 30 sec and 50°C for 15 sec, followed by 60°C for 4 min. Products were purified using the DyeEx Spin column (Qiagen), and analyzed on the ABI Prism 310 Genetic Analyzer (Applied Biosystems). The sequences obtained from the 5 primer sets were aligned to one consensus sequence by SeqScape (Version 1.1, Applied Biosystems) and then compared to reference sequences of the FCoV S-gene deposited in the genebank:

KU2 (D32044) and UCD1 (AB088222) for typical type 1 strains and FIPV79-1183 (X80799) and FIPV79-1164 (X06177) for type 2 strains.

Statistical evaluation

Statistical analyses were performed using StatView 5.1. (SAS Institute Inc., Cary, NC) and Excel Microsoft. To characterize frequencies of antibodies to serotypes with respect to significant differences in the different groups of cats, the χ^2 -test was applied. To evaluate the height of titers in the different groups of cats, titers were divided into two groups (Table 2). The association between the nature of symptoms and the serotypes was evaluated by means of multivariate logistic regression model. To determine the immunological relationship between FCoV 1 and 2 and TGEV, the coefficient of correlation and the regression coefficient using the least square method were applied. To characterize the kinetic of antibody development for significant differences, antibody titers were analyzed by ANOVA, factorial and repeated measures method, and Fisher's PLDS post hoc test for factorial ANOVA. For the comparison of mean titers, the Kruskal-Wallis and the Mann-Whitney-U test were used. Comparison of groups was performed by using the χ^2 -test and the post hoc cell contribution as well as multivariate logistic regression model. Values of $p < 0.05$ were considered statistically significant. For the presentations of frequency distributions in Figures 3 and 4, box-plots were used with the upper and lower levels of the boxes representing the 75th and 25th quantiles, the line in between representing the median and the whiskers the 2.5th and 97.5th quantile. Outliers are shown as individual dots.

Table 2: Assignment of titers to two categories.

Slide type \ Titer group	Low	High
TGEV	1:25 – 1:400	1:1600
FCoV	1:100 – 1:1,600	1:6,400 – 1:409,600

Results

Quality control of the FCoV and TGEV slides

The RT-PCR and PCR testing for potentially contaminating pathogens yielded negative results with the exception of FCoV RT-PCR, which was highly positive (TaqMan Ct values of 18 and 21 for FCoV type 1 and FCoV type 2, respectively, suggesting presence of comparable amounts of viral RNA).

Molecular characterization of the FCoV 1 and 2 isolates

Partial sequencing of the S-gene of FCoV isolated from FIP1 and FIP2 slides (GenBank Accession Numbers DQ122858 and DQ122859 respectively) revealed a high homology with prototypes of FCoV type 1 and 2 strains, respectively (Table 3).

Table 3: Homology of FCoV antigens on IFA slides with FCoV reference strains.

Reference Sequence FCoV slide	FCoV type 1 strains		FCoV type 2 strains	
	UCD 1	KU2	79-1183	79-1164
FCoV slide FIP 1	91.9%	91.8%	62.6%	61.8%
FCoV slide FIP 2	61.8%	62.3%	94.4%	95.3%

Development of antibody titers to FCoV type 1 and 2 during experimental infection

The course of infection was followed in seven cats, experimentally infected with a FCoV type 1 strain, over a period of 40/42 weeks (Figure 1). Antibody titers to type 1 but not type 2 increase significantly after week 17 of the experiment. Of note, the cats survived the experiment and showed no clinical signs; the rise of FCoV 1 antibody titers did not reflect FIP development.

Prevalence of FCoV serotypes in Switzerland

Among 296 clinically healthy cats (Group A), 50% were FCoV seropositive. In 35.1% of the tested animals, titers to FCoV 1 were higher than those to FCoV 2, while in 4.4% of the cats it was vice versa, and 10.5% showed identical titers for both FCoV types (Figure 2).

There was no statistically significant association between the husbandry conditions (multicat vs. single cat household) and the prevalence of FCoV serotypes (χ^2 -test,

$p = 0.07$). High titers ($\geq 1:1,600$) to FCoV were more frequently found in cats from multicat than in single cat households although the difference was not significant (χ^2 -test, $p = 0.06$). Antibodies to FCoV type 2 had a tendency to be less frequent in multicat households (χ^2 -test, $p = 0.07$).

In 15 (71%) of the 21 Swiss catteries (Group B) antibodies to type 1 were predominant, while in 6 (29%) catteries antibodies to type 1 and 2 were equal in height. In none of the catteries antibodies to type 2 were predominant (Figure 2).

In all cats analyzed, antibody titers against FCoV type 1 were significantly higher than against FCoV type 2 (Figure 3).

Association between the FCoV serotype and the clinical manifestation

Among the 500 field sera from 1996/97 (Group A), seropositivity to FCoV (type 1 or type 2) was significantly higher in cats with clinical signs than in healthy cats (χ^2 -test, $p = 0.01$). In this association, antibodies to type 1 were of predominant importance for clinical signs. However, no statistically significant association was found between the presence of antibodies to type 1 and any of the six categories of clinical signs previously found to be associated with possible FCoV infection (multivariate logistic regression model, $p > 0.05$).

All of the 30 samples from histopathologically confirmed FIP cases (Group C) were seropositive. In 25 cats (83%), type 1 titers were higher than those to type 2; the remaining 5 animals (17%) had identical titers to both serotypes. In none of the cats, titers to type 2 were higher than to type 1 (Figure 2). In the reference group of non-FIP cases (Group D), 43% were seronegative, 50% tested higher for type 1 than for type 2, 7% showed identical titers to both types, and in no cat titers were higher to type 2 than to type 1 (Figure 2). The distribution of titers to the FCoV 1 and 2 serotypes did not differ significantly between groups A and D (χ^2 -test, $p > 0.2$). Compared to non-FIP cases, the FIP cases had a significantly higher seroprevalence of FCoV antibodies than any other group (χ^2 -test, $p < 0.001$). In addition, the prevalence of antibodies to FCoV 1 was significantly higher in the FIP group than their reference Group D (χ^2 -test, $p < 0.0001$).

No association could be detected between the height of titer and the type of clinical signs (χ^2 -test, $p > 0.2$). However, mean titers to FCoV type 1 in confirmed FIP cases were statistically higher than to FCoV type 1 and 2 in the control group without FIP (χ^2 -test, $p < 0.0001$, Figure 4).

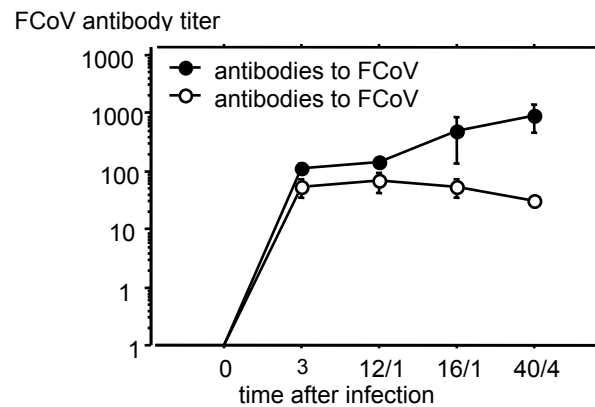


Figure 1. Course of mean titers of antibodies to both FCoV 1 and 2 serotypes in cats experimentally infected with FCoV serotype 1. A statistically relevant specification of the immune system to the FCoV serotype of infection can be seen 40/42 weeks after infection (ANOVA with repeated measures, $p = 0.038$).

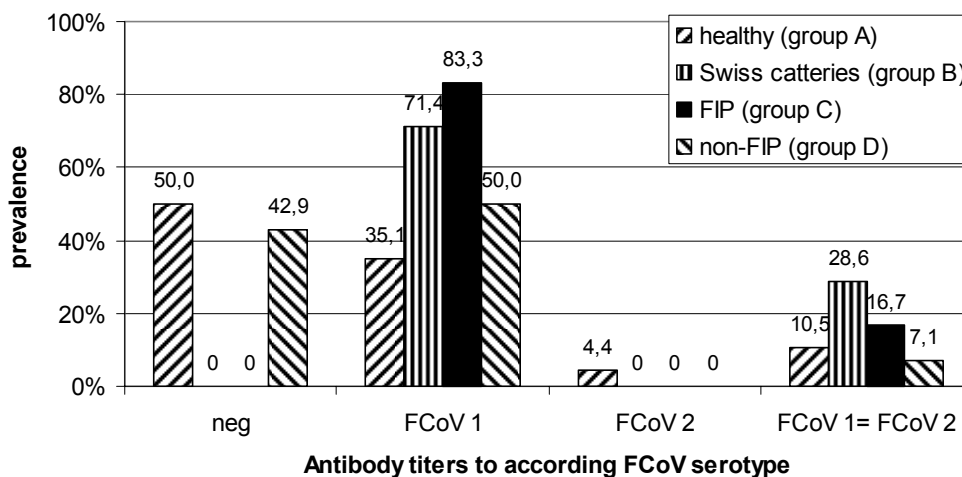


Figure 2. Prevalence of FCoV serotypes in different Swiss cat groups. Numbers on bars indicate the percentage of samples within the according group (A–D) referring to the four categories on the x-axis. Categories are defined as follows: neg: antibody titers to both FCoV serotypes below detectable level; FCoV 1: antibody titers to FCoV 1 > antibody titers to FCoV 2; FCoV 2: antibody titers to FCoV 2 > antibody titers to FCoV 1; FCoV 1=FCoV 2: antibody titers to FCoV = antibody titers to FCoV 2.

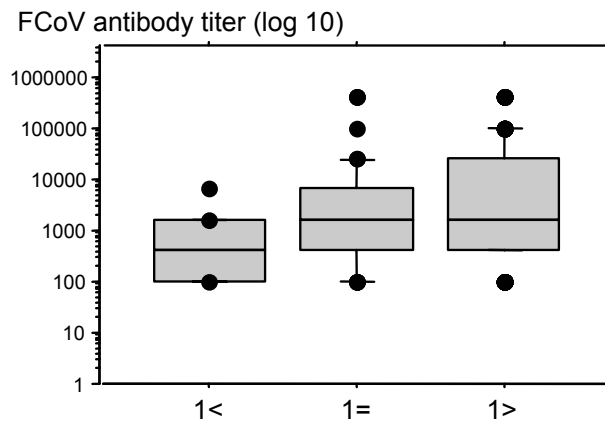


Figure 3. “Box-plot” distribution of titres to FCoV type 1 and 2. In cats with antibodies to FCoV type 1 (1>2), the titres were significantly higher than in cats with antibodies to FCoV type 2 (2>1) and equal titers (1=2; Kruskal-Wallis, $p < 0.0001$).

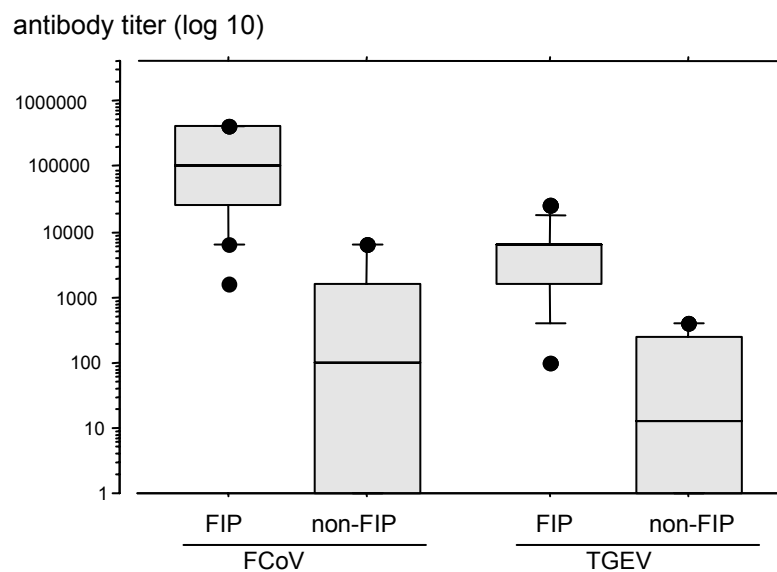
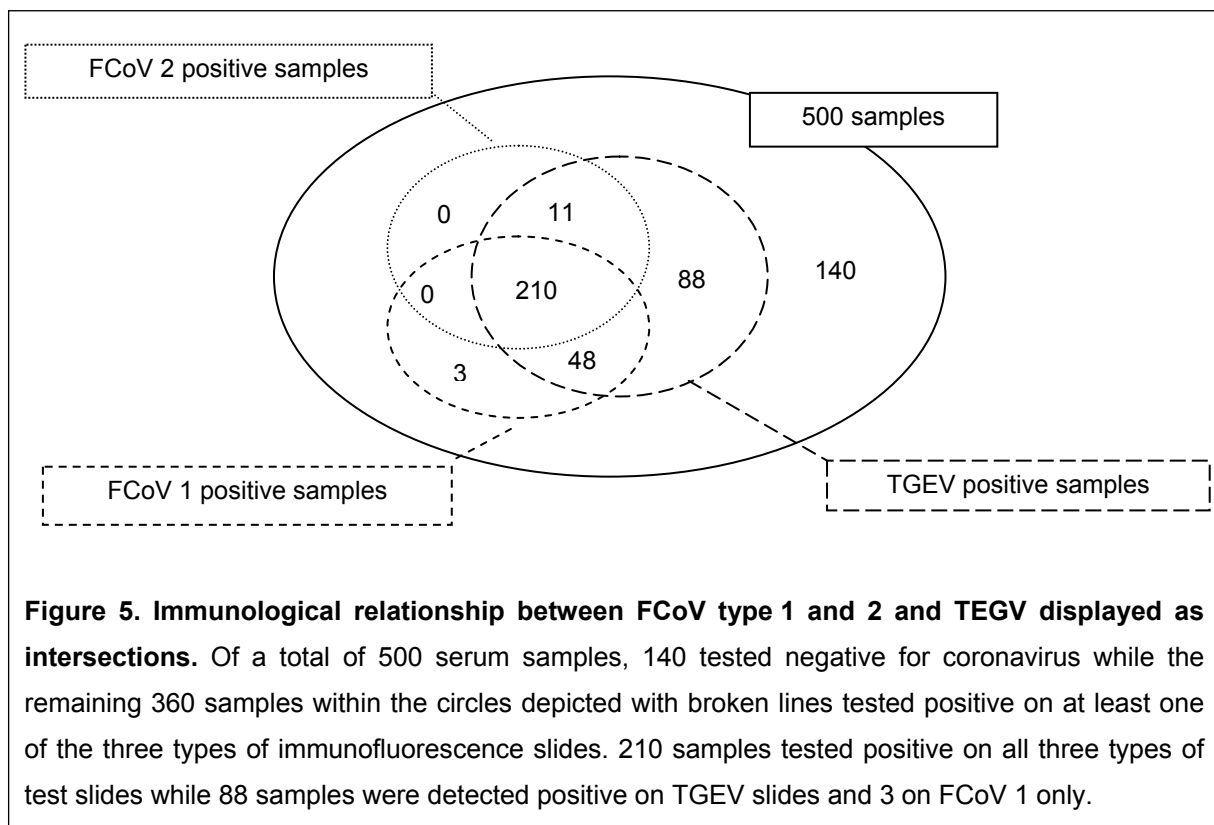


Figure 4. “Box-plot” distribution of FCoV (serotype 1 and 2) and TGEV antibody titres of FIP (group C) and non-FIP cases (group D). FIP cases show significantly higher FCoV/ TGEV antibody titres than non-FIP cases (Mann-Whitney-U, $p < 0.0001$ for both FCoV and TGEV). The ratio of median titers of FIP and non-FIP cases was identical for both, FCoV and TGEV antigen.

Immunological relationship between FCoV type 1 and 2 and TEGV and diagnostic efficiency

The positive correlation of titers between FCoV 1 and 2 ($r = 0.76$, $p < 0.0001$), FCoV type 1 and TGEV ($r = 0.46$, $p < 0.0001$), and FCoV type 2 and TGEV ($r = 0.44$, $p < 0.0001$) confirmed the close immunological relationship of the three antigens tested.

The number of positive IFA results with FCoV 1 was similar to that obtained with FCoV 2 but lower than using TGEV (Figure 5). There were 88 samples that tested positive for TGEV but negative for FCoV; of these samples, 72% had a titer of 25, 27% had a titer of 100, and 1% of 400. When serology in addition to the evaluation of clinical parameters, haematology, and clinical chemistry results was used as an aid to diagnose clinical FIP, TGEV antigen yielded a diagnostic efficiency identical to that of both FCoV serotypes (Figure 4).



Discussion

We have analyzed 667 serum samples from Swiss field cats and experimentally infected cats for FCoV antibodies using FCoV type 1 and type 2 and TGEV slides. We found a prevalence of FCoV 1 antibodies of 68% over FCoV 2 antibodies of 9% while 23% of the cats had equally high titers to type 1 and 2.

Using molecular assays, we demonstrated that the antigen preparations were free of contaminating agents including FeLV, FIV, FHV-1, FPV, and FCV. Based on sequencing analyses of part of the FCoV S-gene, a clear assignment of the two viruses to prototype FCoV 1 and 2 was possible confirming the validity of the slides for serodiagnosis of antibodies to FCoV 1 and 2.

Differentiation of antibodies to type 1 over type 2 occurs late in experimental FCoV 1 infection

When the antibody development to FCoV 1 and 2 was followed in experimentally FCoV 1 infected cats, it became clear that only after an observation period of more than 17 weeks antibodies to FCoV 1 showed a significant increase over those to FCoV 2. It suggests that during early phase of the infection crossreacting epitopes of both types are responsible for comparable titers. From this experiment we conclude that the B-lymphocytes involved undergo constant stimulation resulting in increasing concentrations of antibodies and affinity maturation (30) leading to ever higher antibody specificity to the infecting agent, i.e. to FCoV 1 over FCoV 2. Hence, a serological differentiation between antibodies to FCoV 1 and 2 may not be possible in the early phase of infection but only after the infection has resulted in an increase of antibody concentration and specificity.

Prevalence increase of FCoV serotypes in Switzerland

The prevalence of antibodies to FCoV in the healthy field cats was found to vary between 14.6% in Japan (20) to > 70% in Austria (36). We now report a FCoV seroprevalence of 50% in healthy Swiss cats; this is more than twofold higher than what we had found in a study in 1987 (28). Explanations for this increase include differences in sample selection and/or serologic tests. While in the earlier study TGEV slides were used, we now compared TGEV with FCoV type 1 and 2 slides; all

three antigens gave comparable results. Therefore, the prevalence increase cannot be attributed to the antigens used. In both studies, the criteria for sample submission were identical, i.e. the veterinarians submitted samples from which they had a personal interest in obtaining the results. The two studies were similar with respect to the origin of the samples (single vs. multicat households). Thus, the observed increase may indeed reflect an increase in seroprevalence between 1987 and 1997. We found predominantly FCoV type 1 antibodies (68%); this is in agreement with other serological surveys although the distribution varies to some degree (1, 20, 33, 36, 39). In parallel to our experimental study, we hypothesize that serum samples with antibody titres to FCoV type 1 > type 2 originate from cats that had been FCoV type 1 infected for more than 17 weeks. Field cats with titres to FCoV type 1 equal to type 2 (23%) may represent cats infected with both serotypes or by either virus for a short period. Cats with titres to FCoV type 2 > type 1 (9%) were considered to be infected by a type 2 virus assuming that the antibody development after infection with a type 2 FCoV mirrors that of a type 1 infection with specific antibodies to FCoV type 2 increasing over those to type 1 with time. Overall, it becomes clear that the true prevalence of type 1 and 2 infection cannot be determined precisely as the status of the 23% of cats displaying identical titers to type 1 and 2 remains unresolved.

In an earlier report (16), it was stated that cats infected with FCoV are not readily superinfected by antigenically related coronaviruses. However, this observation could not be confirmed by PCR studies conducted by others (3, 12, 20). Among the cats with identical titers to both serotypes the majority (59%) had high titers ($\geq 1,600$). This may reflect coinfection by both serotypes. Alternatively, it could be explained by an infection caused by a strain not identical but immunologically equally related to both strains used in the current study. Additionally, equal titers to both FCoV types could also be explained by antibodies against other structural proteins present in both viruses such as the M and N proteins.

Based on the assumption that the virus serotypes within a cattery cluster around one strain (2, 16, 26), we tested pooled serum samples of randomly selected Swiss catteries for their predominant serotype. In none of the catteries antibody titers to type 2 were predominant. Along with the low seroprevalence of type 2 in individual cats, these data support the hypothesis that the type typically known as type 2 does not occur very frequently in the field. In spite of this, type 2 viruses are most

frequently used in laboratories. This may not reflect their importance in the field but rather their ease of cultivation *in vitro*.

It has been reported that the type 2 FCoV originate from recombination between coronaviruses of cats and dogs (17). An association between presence of dogs and serotype 2 FCoV could not be confirmed due to the retrospective nature of the study in which no information on the presence of dogs had been recorded. It could be imagined that some of the type 2 viruses have suboptimal replication activities *in vivo* which may explain lower viral loads resulting from infection with these viruses. This may explain the significantly lower antibody titers in cats infected with FCoV type 2 compared with those infected with type 1.

The understanding of the mechanisms of the development of FIP from a FCoV infection is of urgent practical importance to cat owners in terms of husbandry measures. An association between multicat environment and height of antibody titers and the prevalence of FCoV infection was found in our study confirming other reports (9, 28).

Association between the FCoV serotype and the clinical manifestation

Antibodies against type 1 are overrepresented in diseased cats. Additionally, there is a tendency for type 2 to be less often present, however not at a statistically significant level. Similarly, in Japan, 80.4% and 10.1% of chronically diseased cats tested positive for FCoV type 1 and type 2, respectively (20). Thus, FCoV type 1 viruses may be more likely to replicate to high viral load, induce higher antibody titers, and thereby may become more frequently associated with chronic disease and/or FIP. Again, this can be explained by the better adaptation of type 1 to cats. In our study 100% of confirmed FIP cases were seropositive to FCoV. This high seroprevalence in FIP cases was significantly higher compared to the healthy cat population and corresponds well with 87% and 96% found in other studies (6, 32). All of our FIP samples tested positive for type 1 or both types similarly. We could not find a single FIP case testing higher for type 2. Thus, the above mentioned hypothesis of FCoV type 1 being of higher pathogenicity than type 2 appears to be true also for FIP development.

Although confirmed FIP cases showed significantly increased antibody titers to FCoV, no significant association could be found with height of titers and the category of clinical signs.

Immunological relationship between FCoV type 1 and 2 and TGEV and implication for serotesting

The statistically significant correlation of antibody titers to FCoV 1 / FCoV 2, FCoV 1 / TGEV, and FCoV 2 / TGEV suggest close immunological relationship. From Figure 5 it becomes evident that TGEV as IFA substrate allowed detection of 88 cats to be seropositive which would not have been detected using FCoV 1 or FCoV 2 antigens. The low titers of these samples (73% with titres of 25 and 27% with titres of 100) suggest that TGEV may be a better substrate, i.e. produces higher concentrations of viral antigens in its pig cell line than FCoV 1 and 2 grown in cat cells. Alternatively, the antibodies present in these 88 samples could have been induced by a third type of FCoV currently not known which is more closely related to TGEV than FCoV. For the diagnosis of clinical FIP (37), TGEV is equally suited as FCoV. Therefore, for FIP diagnosis, either FCoV or TGEV can be used. It is emphasized, however, that coronavirus titres alone must not be used as single parameter for FIP diagnosis (23). However, if seropositivity is to be detected, TGEV in pig cells appears to be better suited.

In conclusion, it was demonstrated that FCoV type 1 is the most prevalent FCoV serotype in Switzerland and with this the distribution is quite similar to that in other parts of the world. This information may be important for the development of future FCoV vaccines. Differentiation of the antibody response to FCoV 1 and 2 does not seem to be very helpful to obtain information of the disease process in a given cat. However, the antigens used in this study proved to be highly valuable for the differentiation of antibodies with specificity for FCoV 1 and 2.

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